

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 24 December 1998	3. REPORT TYPE AND DATES COVERED Final Project Report	
4. TITLE AND SUBTITLE Potential For Using Biogeochemical Markers To Assess And Monitor The Impact Of Military Training Exercises On Desert Ecosystems: Proof-Of-Concept			5. FUNDING NUMBERS N/A	
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) SERDP 901 North Stuart St. Suite 303 Arlington, VA 22203			10. SPONSORING / MONITORING AGENCY REPORT NUMBER N/A	
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14. SUBJECT TERMS military training exercises; desert ecosystem; nitrogen fixation; nitrogen flux; SERDP; SERDP Collection				15. NUMBER OF PAGES 13
				16. PRICE CODE N/A
17. SECURITY CLASSIFICATION OF REPORT unclass.	18. SECURITY CLASSIFICATION OF THIS PAGE unclass.	19. SECURITY CLASSIFICATION OF ABSTRACT unclass.		20. LIMITATION OF ABSTRACT UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

19990521 118

**Potential For Using Biogeochemical Markers To Assess And Monitor The Impact
Of Military Training Exercises On Desert Ecosystems: Proof-Of-Concept**

Final Project Report
SERDP Project CS 1101

24 December 1998

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Potential For Using Biogeochemical Markers To Assess And Monitor The Impact Of Military Training Exercises On Desert Ecosystems: Proof-Of-Concept

ABSTRACT

The rate of nitrogen fixation and nitrogen flux in soil samples collected from a pristine and heavily disturbed site at MCAGCC 29 Palms were 6 ± 2.05 and 3 ± 1.04 mM ethylene/g-h and 30.1 ± 6.47 and 21.9 ± 6.95 $\mu\text{g}/\text{m}^2 - \text{d}$ respectively ($P \leq 0.05$). In addition, significantly more bacteria are present in the disturbed soil samples and principle component analysis demonstrates distinct differences in the bacteria at each site. These data can be interpreted in terms of the destruction of vegetation and release of nutrients that accompany military training exercises. Decay of damaged vegetation releases nutrients that support bacterial growth and suppresses nitrogen-fixing bacteria. The bacteria at the disturbed site also appear to be experiencing an increase in environmental stress that may be associated with the damaged vegetation, soil compaction, reduced percolation of water, and/or a decrease in oxygen in the soil gas. A solid state ammonia sensor constructed by American Research Corporation of Virginia and tested in desert soil detected ammonia concentrations as low as 20 ppb which is the concentration reported for desert soils in this and other studies.

INTRODUCTION

Desert land DOD facilities encompass more than two million acres and provide a realistic training environment that is essential for maintaining military readiness. These include Navy and Marine Corps facilities at MCAGCC 29 Palms, CA (595,367 acres), MCB Camp Pendleton, CA (125,000 acres), NAWS China Lake, CA (608,190 acres), Yuma MCAS, AZ (3,000 acres) and the associated Chocolate Mountain Aerial Gunnery Range at Niland, CA (460,000 acres), MCLB Barstow, CA (5,688 acres), Fallon Naval Air Station, NV (7,982 acres), and the Army National Training Center, Fort Irwin, CA (640,000 acres).

However, desert ecosystems are extremely sensitive to disturbances, slow to recover, and they support unique organisms including threatened and endangered species. These concerns have resulted in a number of legislative initiatives aimed at protecting and preserving desert ecosystems. As a result, the DOD as stewards of these lands which also serve as de facto nature preserves has taken a proactive approach to the management of base ecosystems. Examples of this approach are DOD participation in the Mojave Ecosystem Database Program (MEDP) and SERDP sponsored land management research projects, e.g., the Army's MIMs (Maneuver Impact Miles) and Integrated Dynamic Landscape Analysis and Modeling System (IDLAMS). The objective of these programs is to develop tools that can be used to assess and manage training associated ecological impact.

The immediate and inevitable consequences of desert training impacts are the loss of plants, cryptogamic communities, and soil compaction. Since these impacts have been

identified as the primary determinants of arid land degradation (Ray, 1995), the quantification of these events as land management tools is being pursued. However, the heterogeneity and dynamic nature of desert ecosystems including seasonal variability and successional changes brought about by fires, storms, and disease complicate the interpretation of these results (Schimel, 1995). Thus, there is a critical need for assessment tools that provide a real-time response to disturbance associated ecosystem impacts from which seasonal, spatial, and temporal variation can be filtered. An additional benefit would be the capability to provide ground-truth for approaches that use remote sensing.

Despite the seemingly impossible complexity that this requirement poses, Schlesinger (1991) and Schlesinger et al. (1990) have identified the interdependence of the biogeochemical cycles of carbon and nitrogen as a key element in assessing desert ecosystems. The biogeochemical cycles are the processes that shuttle elements between their inorganic (primarily CO_2 and N_2) and organic (for example, proteins and nucleic acids) forms. The cycles are tightly coupled due to the carbon nitrogen stoichiometry which regulates the transfer of these elements within and between autotrophs (producers) and heterotrophs (consumers) and the overall regulation of these processes by water (Collatz et al., 1991).

Thus, selected aspects of the biogeochemical cycles of nitrogen and carbon and the structure of the bacterial community associated with them should be sensitive indicators of the ecological impact of training exercises. For example, Harrison et al. (1993) have shown that one of the consequences of agriculture induced disturbance of soil ecosystems is the loss of what they refer to as the fast turnover time pool of humic materials. Although the data are for agricultural soils, the research demonstrates how large-scale disturbances are correlated with an essential soil component that is derived from biogeochemical cycling in the soil. Thinner and less robust desert soils appear to even more susceptible to these types of disturbances.

Training associated destruction of plants and resultant decay releases carbon, phosphorus, and nitrogenous compounds to the soil. This influx of nutrients may initiate a shift in the microbial population of the soil, from nitrogen fixation and carbon storage to nitrogen release and carbon use. Klein et al. (1996) detected these types of responses in test plots that were amended with nitrogen. Additional studies have shown that adding nitrogen and carbon to an ecosystem affects succession and reestablishment of the plant community (McLendon and Redente, 1991; McLendon and Redente, 1992; Redente et al., 1992). Critical determinants of these processes are the nitrogen content (Mosier et al., 1991) and the phosphorus nitrogen ratio (Eisele et al., 1989). These processes are also sensitive to diffusive gas transport and the water content of the soil matrix (King and Adamsen, 1992), properties that are directly impacted by soil compaction associated with vehicle and troop traffic.

When military training impacts lead to plant loss, the rhizosphere bacterial communities are also greatly diminished or lost. The loss of root zone associated bacteria that are

known to play an important role in nitrogen cycling and by association carbon cycling coupled with the spatial heterogeneity of desert ecosystems (Lajtha and Schlesinger, 1988; Skuji, 1984) may contribute to the slow recovery of desert ecosystems.

A particularly important aspect of the relationship between bacteria and plants is the biogeochemical cycling of nitrogen (Carter et al., 1995; Lajtha and Schlesinger, 1986; Herman et al., 1993; Hobbs et al., 1991). Plants also excrete compounds that promote the growth of beneficial bacteria and inhibit bacteria that may be detrimental (reviewed by Rovira, 1989). In all terrestrial ecosystems, numerous other bacteria essential to the health of the plants are closely associated with the plant root zone, (Newman, 1985; Parkin, 1993; Smith and Tiedje, 1979; Turner and Newman, 1984).

In training impacted desert soils, loss of plant cover and reduced percolation in compacted soils is associated with water stress (Pappendick and Campbell, 1981). In the saline alkaline desert soils typical of MCAGCC 29 Palms, changes in the availability and quality of water may trigger changes in pH (Hall et al., 1995) and salinity (Galinski, 1995), both of which are known stressors.

As the effects of large-scale disturbances propagate through the ecosystem, they may lead to loss of species (biodiversity) and diversity within a species (genetic diversity). At the microbial level this includes groups of bacteria generically referred to as nitrogen fixing bacteria, methanotrophs, and nitrifiers, that perform essential functions related to the cycling of nitrogen and carbon. Loss of biodiversity and/or shifts in bacterial populations are linked to changes in the fluxes of nutrients and pollutants, and the stabilization of the physical environment. Consequently, disruption of the ecosystem at the microbial level is an unseen but insidious consequence of impacts on higher organisms. As a result, the overall health of the ecosystem may be compromised, higher plant and animal species are threatened, and the recovery of the ecosystem may be impeded. However, conducting a detailed biogeochemical assessment whenever training is scheduled is not practical.

The need for real-time data can be met by using sensors that measure biogeochemical associated processes directly, e.g., the concentration of ammonia and correlated with more detailed measurements derived from lab and field data. Alternatively, some parameters, e.g., soil compaction caused by vehicles and troops might be measured with pressure transducers and subsequently correlated with biogeochemical changes measured in the lab and field. Furthermore, sensors can be queried from remote locations, and the data incorporated directly into GIS based management tools supported by SERDP, e.g., IDLAMS and they provide ground truth for air-based remote sensing.

PROOF-OF-CONCEPT

In response to concerns raised by SERDP in response to the presentation of the full proposal at the SERDP SAB meeting in September 1997, the following series of experiments, i.e., proof-of-concept, were proposed on 10 October 1997. This protocol was accepted and funded by SERDP with a start date of 1 January 1998. The

experimental work was completed in April and presented to the SERDP at the May in-progress review.

Proposal: Investigate the potential for using biogeochemical characteristics of the Mojave Desert Ecosystem at MCAGCC 29 Palms as one measure of the impact of military training exercises. Once developed, these measurements have the potential to be used in conjunction with a variety of tools, e.g., soil erosion modeling that are being developed in other laboratories for monitoring and assessing the impact of military training exercises on a variety of ecosystems.

Assumption: Training impacts are associated with detectable changes in biogeochemical parameters. To demonstrate this concept, laboratory experiments will focus on nitrogen fixation, ammonia flux, bacterial community structure, and chemical composition of soil collected at MCAGCC 29 Palms.

Soil. Three soil samples (six total) will be collected from impacted and non-impacted areas at MCAGCC 29 Palms and the following experiments performed in triplicate on each soil sample. Since the intent is to demonstrate the validity of using biogeochemical assessments, adequate data will require this level of sampling and experimentation. The soil will be collected from sites identified by Sharon Jones and her colleagues at MCAGCC 29 Palms.

Experiment 1. Bacterial community structure. Extract bacterial lipids from soil samples, convert to their methyl ester derivatives, chromatograph and compare the fatty acid profiles using principle component analysis (PCA).

Rationale: Disturbance events cause the disruption and re-establishment of bacterial communities that can be distinguished by their fatty acid profiles.

Experiment 2. Rate of nitrogen fixation. Soil samples will be incubated with acetylene in closed containers. At regular intervals the headspace will be sampled and chromatographed to detect ethylene. The results will be expressed as millimoles of nitrogen fixed per day per gram of soil (dry weight).

Rationale: Impacted and non-impacted lands exhibit nitrogen-dependent differences in biological community succession. Thus, impacted and non-impacted soils would be expected to exhibit differences in the rate and quantity of nitrogen fixed.

Experiment 3. Solid state ammonia sensor performance. Place the solid state ammonia electrode developed by American Research Corporation of Virginia (ARCOVA) in soil and determine the dynamic sensing range of the electrode. Various configurations of the sensor being developed by ARCOVA will be tested.

Rationale: Demonstrate that a solid state sensor can be used to measure the flux of ammonia when placed in the soil. This is a critical point since we propose to use these types of sensors to collect real time data for ecosystem management.

Experiment 4. Rate of ammonia flux. Measure the rate of ammonia flux from soil samples by trapping with sulfuric acid, performing a Kjeldahl digestion and assay for ammonia.

Rationale: The Kjeldahl method is an accepted method for ammonia determinations and will be performed to confirm the results of the previous experiments and serve as an independent confirmation of the ammonia electrode results.

Experiment 5. Extract soil samples with chromatography grade water and use ion chromatography to analyze for major/minor cations and anions. Concentrations will be expressed as millimoles per gram of soil (dry weight).

Rationale: While the focus of the previous experiments is nitrogen, disturbance events may lead to changes in other chemical species. Exploratory analyses are necessary to investigate and identify biogeochemical parameters that can be used for ecosystem assessment.

The research reported was conducted in collaboration with American Research Corporation of Virginia which is developing sensors that are inexpensive and can withstand the rigors of the training environment. These sensors use a laser diode and cost \$20 - \$50. By modifying the surface, they can be tailored to respond to a variety of chemical species. For this phase of the project, ARCOVA developed and tested an ammonia sensor.

METHODOLOGY

Sites and Sampling. An undisturbed pristine site that served as the control is shown in Figure 1, [Phospholipids\Figure 1.tif](#), and a sampling point is shown in Figure 2, [Phospholipids\Figure 2.tif](#). An area that is used for tank training exercises was used as a disturbed area Figure 3, [Phospholipids\Figure 3. Disturbed.tif](#). The mound in the background with PVC pipes inserted in it is a creosote revegetation project.

Since the focus of this phase of the project is the subsurface, samples were taken from a depth of 2-6 inches following removal of the top one inch of soil. Three samples were collected at each site and all analyses were run in triplicate on each sample. Average values were calculated and compared using the Students T-Test to calculate a two tailed P value. Soil collected from a third site that has not been used for training for several years was sent to American Research Corporation for testing the ammonia sensor.

Nitrogen Fixation. Soil samples were incubated in serum bottles containing acetylene, and the headspace sampled and chromatographed to quantitate ethylene (Balderston et al., 1976; Bedard and Knowles, 1989; Fireston and Davidson, 1989; Tiedje, 1988).

Phospholipids. Gas chromatography of lipids extracted from soil samples was used to estimate microbial biomass (Vance, et al., 1987; Zelles et al., 1994; Zelles et al., 1995), the distribution of eukaryotes and prokaryotes, and as indicators of the stress experienced by the gram-negative bacteria (Balkwill, et al., 1988; Frostegard et al., 1991; Gillan and Hogg, 1984; Guckert et al., 1985; Peterson and King, 1994; Vestal and White, 1989). Atlas (1984) first described changes in microbial phospholipids in response to a variety of stressors. Numerous additional examples (Brown, 1990; Frostegard, Baathe, and Tunlid, 1993a; Frostegard, Tunlid, and Baathe, 1993b; McKinley et al., 1984) have demonstrated the value of this method for assessing the status and composition of the indigenous microbial community in response to environmental insults. This work was conducted in collaboration with Greg Davis at Microbial Insights, Inc.

Microbial Enumeration. Total heterotrophs were enumerated using the fifteen tube most probable number method in a basal salts medium developed specifically for growing and enumerating bacteria at Fallon Naval Air Station and MCAGCC 29 Palms and used in previous bioremediation work, Table 1, Phospholipids\FSM.DOC. To enumerate heterotrophs, the medium was amended with 0.025% (w/v) each of yeast extract and casamino acids.

Ammonia Sensor. Construction and testing of the ammonia sensor was conducted by American Research Corporation of Virginia. The test system is illustrated in Figure 4, Phospholipids\NH3 Annalyzer.doc and the ammonia sensor is illustrated in Figure 5, Phospholipids\NH3 Detector.doc.

RESULTS AND DISCUSSION

The size of the microbial population in each soil sample was estimated from the total mass of extracted phospholipid fatty acids (PLFA). These data are shown in Figure 6 Phospholipids\Biomass content of soil.doc and suggest a larger microbial population in the soil samples from the disturbed site, i.e., 186349 picomoles PLFA from the disturbed site vs 11091 picomoles PLFA from the pristine site. When heterotrophs were enumerated in these soil samples using the MPN method, the average number of heterotrophs in the disturbed and pristine samples were $1.5 \times 10^7 \pm 2.1 \times 10^6$ and $1.04 \times 10^7 \pm 2.1 \times 10^6$ bacteria/g soil (dry weight), $P = 0.0478$ respectively. These data support the idea that release of nutrients associated with the destruction of the vegetation supports an increase in the bacterial population.

An analysis of fatty acids characteristic of the growth phase of gram-negative bacteria also supports the proposal that the release of nutrients associated with the destruction of plants at the disturbed site promotes bacterial growth, Figure 7, Phospholipids\Growth Phase.doc. However, in the samples from the disturbed site there is an increase in PLFAs

characteristic of gram-negative bacteria exposed to environmental stressors, Figure 8, Phospholipids\Stress.doc. This observation is consistent with the proposal that soil compaction, destruction of plants and other disturbance events stress the microbial ecosystem.

Characteristic phospholipids were used to estimate the microbial composition of each soil sample Figure 9, Phospholipids\Soil Biota.doc. These data suggest a fairly uniform distribution of prokaryotes and eukaryotes among all the samples but (see the legend to Figure 9) there are significantly fewer prokaryotes and eukaryotes at the pristine site. However, the dominant gram-negative bacteria belong to the genera Actinomycetes and Desulfovibrio. The former organisms are common in soil and sulfate-reducing bacteria would be expected in the saline sulfate rich desert soils. However, principle component analysis Figure 10, Phospholipids\PCA Analysis.doc of these data demonstrate that there is a distinctly unique distribution of bacteria at each site.

Additional differences between the soil samples were found in the flux of ammonia from the soil, Figure 11, Phospholipids\Ammonia flux.doc and nitrogenase activity, Figure 12, Phospholipids\Nitrogenase Activity.doc. The lower flux of ammonia from the impacted soil is consistent with the increase in biomass which would be expected to capture the ammonia for use as a molecular building block. The availability of ammonia in the disturbed site would also be expected to suppress nitrogen fixation which is supported by the data in Figure 12. Analysis of soil samples also supports the differences in the availability of nitrogen at the disturbed site compared to the pristine site, Table 2, Phospholipids\average values.doc.

Performance of the solid state ammonia sensor when tested with 40 ppb and 20 ppb of ammonia are shown in Figure 13, Phospholipids\Ammonia 40 ppb.doc and Figure 14, Phospholipids\Ammonia 20 ppb.doc respectively. As the ammonia passes through the column, the concentration of may be reduced due to adsorption and chemical reactivity. Thus, the concentration at the sensor may be less than the input concentration. However, the response of the sensor to 20 ppb ammonia is within the range of ammonia flux expected from desert soils, this study Figure 11 and Schlesinger et al. (1990).

CONCLUSIONS

The data support the premise in the original proposal and the scaled down proof-of-concept which is that biogeochemical markers can be used to monitor the ecological impact of military training. Specifically, the data presented here confirm that there are measurable and statistically significant ($P \leq 0.05$) differences in the flux of ammonia and rate of nitrogen fixation between an impacted and pristine site.

These data can be interpreted in terms of the destruction of vegetation and release of nutrients. Thus, there is the potential for correlating these types of measurements with remote monitoring of vegetation cover. In addition, the data suggest significant differences in the composition of the microbial population at the two sites which may be

associated with additional biogeochemical differences and/or correlated with compaction, soil moisture, and/or oxygen concentration in the soil as discussed in the original proposal. Furthermore, the performance of the solid state ammonia sensor demonstrates the potential for using this technology to monitor and collect real time data from large areas.

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